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PREDICTIVE VALUE OF ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODIES (ANCA) IN SMALL VESSEL VASCULITIS

THESE

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par

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Résumé

Valeur prédictive des anticorps dirigés contre le cytoplasme des neutrophiles (ANCA) dans les vasculites des vaisseaux de petit calibre

But du travail :
Les vasculites sont des pathologies le plus souvent sévères et parfois létales ; elles nécessitent une reconnaissance et un traitement précoces. Il est donc utile de pouvoir disposer de marqueurs diagnostiques, et éventuellement de marqueurs qui puissent prédire l'activité de la maladie.
Les « antineutrophil cytoplasm antibodies » (ANCA) constituent une famille d'auto-anticorps dirigés contre des antigènes du cytoplasme des neutrophiles, cellules clés du processus inflammatoire au cours des vasculites. De nombreuses études ont tenté de préciser l'utilité des ANCA dans le diagnostic et le suivi des vasculites avec des résultats contradictoires. Le but de ce travail a été de passer en revue l'évolution clinique des patients suivis dans notre service pour une vasculite à ANCA et évaluer la valeur prédictive des ANCA comme marqueur de récidive.

Méthode:
Les dossiers médicaux de 36 patients, suivis à notre consultation ambulatoire d'immunologie et allergie du CHUV pour une vasculite à ANCA entre janvier 1990 et décembre 2001, ont été analysés de manière rétrospective afin d'établir une base de données. Les données démographiques, le type de vasculite (granulomatose de Wegener ou polyangéite microscopique) et ses caractéristiques (organes touchés), les traitements reçus, les dosages des ANCA (par immunofluorescence et par ELISA avec détermination des anti-PR3 et/ou anti-MPO), et l'évolution clinique (récidive/rémission) ont été considérés.
La valeur pronostique des ANCA dans notre population a été calculée utilisant les valeurs prédictives positive et négative, la *likelihood* et les *odds ratios*. La valeur statistique a été examinée par les tests Chi-square test ou Fisher's exact test (valeur significative définie comme $p < 0.05$) à l'aide du programme GraphPad Instat software version 3, San Diego, CA.

Résultats :
Vingt-trois patients atteints d'une maladie de Wegener et treize d'une polyangéite microscopique ont été suivis pour une durée médiane de cinq ans (entre 1 mois et 16 ans). La plupart des patients ont été traités avec des corticostéroïdes associés à du cyclophosphamide. Une rémission a été obtenue chez 21 patients (91%) atteints d'une maladie de Wegener, mais 74% ont présenté par la suite une récidive. Tous les patients atteints d'une polyangéite microscopique sont entrés en rémission et 33% ont par la suite récidivé. Une élévation persistante (définie comme supérieure à 6 mois) des ANCA ne s'est pas révélée associée à un risque statistiquement significatif de récidive ($p=0.14$). En revanche, une élévation soudaine du taux des ANCA s'est démontrée prédictive d'une récidive (table 3).

Conclusion :
Durant le suivi de certaines vasculites, comme la granulomatose de Wegener et la polyangéite microscopique, une élévation des ANCA doit faire redouter une exacerbation de la maladie et, par conséquent, justifie une surveillance accrue.

Predictive Value of Antineutrophil Cytoplasmic Antibodies in Small-Vessel Vasculitis

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ABSTRACT. ***Objective.*** The predictive value of antineutrophil cytoplasmic antibodies (ANCA) as markers of clinical activity of small vessel vasculitis is controversial. We reviewed the outcome of patients with ANCA-associated vasculitis from a single center and evaluated the predictive value of ANCA as markers of relapse.

Methods. The medical history of all consecutive patients with ANCA-associated vasculitis followed at our outpatient clinic was retrospectively reviewed. ANCA were monitored by immunofluorescence and by ELISA (antiproteinase 3 and antimyeloperoxidase).

Results. Twenty-three patients with Wegener's granulomatosis and 13 with microscopic polyangiitis were followed for a median period of 5 years (1 mo–16 yrs). Most patients were treated with combined corticosteroids and cyclophosphamide. In the Wegener's granulomatosis group, remission was obtained in 21 of 23 patients (91%), within 4–48 weeks (median 7.5); relapses occurred in 74%. In the microscopic polyangiitis group, remission was obtained in all patients within 3–38 weeks (median 8); relapses occurred in 33%. In contrast to persistently (> 6 mo) elevated ANCA titers, which were not significantly associated with disease relapse, the predictive value of an acute rise in ANCA titers was strongly associated with the magnitude of the increase.

Conclusion. Our study demonstrates the weak predictive value of persistently elevated ANCA titers, in contrast to acute rises in ANCA titers. Although an acute rise in ANCA titers may help in a decision whether to introduce immunosuppressive therapy, the final decision has to be based on both clinical and laboratory markers. (J Rheumatol 2005;32:2167–72)

Key Indexing Terms:
ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES VASCULITIS PREDICTIVE VALUE
WEGENER'S GRANULOMATOSIS MICROSCOPIC POLYANGIITIS RELAPSE

Antineutrophil cytoplasmic antibodies (ANCA) are autoantibodies directed against the cytoplasmic granules of neutrophils and monocytes. ANCA were first reported in 1982 in 8 patients with segmental necrotizing glomerulonephritis[1]. They are essential markers and contribute to classification of small vessel vasculitis, such as Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), and renal-limited vasculitis, which together are categorized as ANCA-associated systemic vasculitides (AAV)[2-6]. The Chapel Hill criteria[7], which do not yet take into account ANCA positivity and antigen specificity, are currently under revision. In patients with WG, ANCA display a diffuse cytoplasmic pattern (cANCA) when detected by indirect immunofluorescence (IF) on ethanol-fixed neutrophils, and are usually directed against proteinase 3 (PR3)[8,9]. The use of indirect IF or ELISA alone resulted in unsatisfactory diagnostic specificity; however, the combination of IF with PR3- and myeloperoxidase (MPO) ELISA showed a 99% specificity for diagnosis of AAV[2,6]. ANCA sensitivity in WG has been reported to be in the range of 34%–92%[3]. Despite the great variability of the studied populations, c-ANCA/anti-PR3 specificity (proportion of patients without WG who have a negative test result) is in the range of 80%–100%[3]. In MPA patients, ANCA display a perinuclear pattern (pANCA) and usually target MPO[9]. The proportion of MPA with positive pANCA/MPO is reported to be in the range of 40%–80%[4].

Immunosuppressive therapy for AAV has considerably reduced mortality, but morbidity remains high because of frequent relapse. In view of the high relapse rate of WG despite optimal immunosuppressive therapy, early detection and prediction of relapse is crucial. The clinical utility of serial measurements of ANCA has been examined by several groups, but their value in monitoring disease activity remains controversial. The sensitivity of a rise in ANCA for the diagnosis of relapse appears to be as low as 24% and as high as 100%[5,10-21]. Different definitions of disease activity, study designs, methods, and timing of ANCA testing may explain in part the high variability of results.

In this retrospective study, we reviewed response to therapy and relapse rate in our series of patients with WG and

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MPA, and evaluated the value of serial ANCA titer measurement in predicting relapse.

MATERIALS AND METHODS

Patients. The medical history of 36 consecutive patients (21 men, 15 women) with WG or MPA was reviewed retrospectively. Median age was 56 years old (range 17–82). Biopsy-proven vasculitis was demonstrated in 32 cases. In 4 patients with a clinical presentation and laboratory findings that strongly suggested diagnosis of vasculitis, treatment was started without invasive biopsy procedures. Patients were classified in 2 groups according to the Chapel Hill criteria[7]: 23 were diagnosed as WG and 13 as MPA. All patients were followed for a median period of 5 years (range 1 mo to 16 yrs).

Vasculitis remission and relapse. Patients were considered to be in remission when the following criteria were fulfilled[20]: (1) return to good general condition; (2) absence of manifestations of AAV; (3) normal erythrocyte sedimentation rate (ESR) and/or C-reactive protein (CRP). Relapse was defined as the reappearance of AAV related symptoms and worsening of general condition, justifying treatment adjustment.

ANCA assays. ANCA were screened by IF (Nova Lite™ ANCA, Inova Diagnostics, San Diego, CA, USA) then titrated by ELISA for anti-MPO and anti-PR3 antibodies (Quanta Lite™, Inova Diagnostics). An acute rise in ANCA titer (as measured by ELISA) was defined as an increase in the most recent sample titer of ≥ 1.2-, 1.5-, 2-, 3-, or 4-fold, resulting in a titer ≥ 20 U[19]. An acute rise in ANCA titer was considered to indicate clinical relapse when occurring within 6 months of the relapse or at the time of the event. Persistently positive ANCA titers were defined as steadily elevated titers ≥ 20 U for more than 6 months[5,19,21]. Persistently positive ANCA titers were considered to indicate clinical relapse when relapse occurred during the period of positive ANCA titers.

Statistical methods. The prognostic value of persistently elevated ANCA titers or acute rises in ANCA at various levels was assessed by measuring positive and negative predictive values, and likelihood and odds ratios. Statistical significance was examined by the chi-square test or Fisher's exact test. P values were judged significant when they were 0.05 or less. Statistical analysis was done using GraphPad Instat software version 3 (Instat, San Diego, CA, USA).

RESULTS

Patient characteristics. Among the 23 patients with WG, 96% (22/23) were positive for cANCA, 4% (1/23) for pANCA, 78% (18/23) for anti-PR3 antibodies, and 4% (1/23) for anti-MPO antibodies. Among the 13 patients with MPA, 85% (11/13) were positive for pANCA, 15% (2/13) for cANCA, 61% (8/13) for anti-MPO antibodies, and 7.7% (1/13) for anti-PR3 antibodies.

At diagnosis, renal, pulmonary, and joint manifestations were the most frequent features in both groups, with associated ear, nose, and throat manifestations in the WG group and neurological manifestations in the MPA group. In a comparison using chi-square test, the profile of target organ manifestations in both diseases differed significantly (Table 1; chi-square test, p = 0.02). At diagnosis, median serum levels of CRP and ESR were 104 mg/l (25%–75% percentile range: 25–170) and 90 mm/h (25%–75% percentile range: 65–115) in the WG group, 59 mg/l (25%–75% percentile range: 53–125) and 84 mm/h (25%–75% percentile range: 58–100) in the MPA group.

Therapy. As remission induction therapy, 21 patients (58%) were treated with intravenous (IV) corticosteroids (methylprednisolone 500 mg/day for 3 days) and all received tapered oral prednisone (starting with 1 mg/kg). Thirty-two patients (89%) received cyclophosphamide: 6/36 (17%) oral[22], 27/36 (75%) IV regimen[23] (a single patient received cyclophosphamide first orally, then by IV). Two patients (one with WG limited to the oropharyngeal area and one with MPA) were treated with oral corticosteroids and methotrexate. Another patient with WG limited to upper respiratory airway and joint involvement was treated with oral corticosteroids only. A fourth patient, diagnosed as MPA with peripheral nervous system involvement, was treated with oral corticosteroids and azathioprine. Three patients required short term and 3 others longterm dialysis, of whom 2 subsequently underwent kidney transplantation.

Table 1. Patient characteristics at diagnosis.

	Wegener's Granulomatosis, n = 23	Microscopic Polyangiitis, n = 13
Male/female ratio, n (%)	15/8 (65)	6/7 (46)
Age, median, range, yrs	56 (17–82)	63 (46–82)
Organ system involvement, n (%)		
Kidney	18 (78)	8 (62)
Lung	14 (61)	4 (31)
Ear, nose, throat	14 (61)	0 (0)
Joints	12 (52)	4 (31)
Skin	1 (4)	3 (23)
Eyes	5 (22)	2 (15)
Nervous system	6 (26)	6 (46)

Remission. According to criteria defined in Materials and Methods, remission was obtained in 21/23 patients (91%) in the WG group in 4–48 weeks (median 7.5). In the MPA group, remission occurred in all patients within 3–38 weeks (median 8).

Relapse. To keep our analysis of relapse rate clinically relevant, only patients followed for a minimum of 11 months were considered (19 WG, 9 MPA). Over a mean followup of 5 years, a total of 21 relapses occurred in 14 of 19 patients (74%) with WG [one relapse in 7 patients (37%), 2 relapses in 6 patients (31%), and 4 in one patient]. Time to first relapse was 15 months (range 2–32 mo). In the MPA group, a total of 4 relapses occurred in 3 of 9 patients (33%; one relapse in 2 patients, and 2 in one patient). Time to first relapse was 36 months (range 32–41). Clinical characteristics of patients at the time of first relapse are shown in Table 2. Relapse incidence in the WG group was significantly higher than in the MPA group (clinical presentations with or without relapse in WG vs MPA; Fisher's exact test, p = 0.039). Relapses were initially treated in 6 of the 17 patients (35%) with IV corticosteroids (methylprednisolone 500 mg/day for 3 days) and all 17 patients received tapered oral prednisone (starting with 1 mg/kg) for at least 6 months.

Table 2. Clinical manifestations at first relapse.

	Wegener's Granulomatosis, n = 14	Microscopic Polyangiitis, n = 3
Male, n (%)	11 (79)	1 (33)
Organ system involvement, n (%)		
Kidney	4 (29)	1 (33)
Lung	7 (50)	1 (33)
Ear, nose, throat	4 (29)	0 (0)
Joints	4 (29)	1 (33)
Skin	0 (0)	0 (0)
Eyes	1 (7)	0 (0)
Nervous system	3 (21)	3 (100)

Twelve patients (71%) received cyclophosphamide (oral 2, IV 10), 2 methotrexate, one azathioprine, and one mycophenolate mofetil as maintenance therapy for one year. Immunosuppressive therapy for relapse was introduced, not based on ANCA titers, but based on the level of clinical disease activity. Among the 17 patients with first relapse, 5 were still under induction phase therapy when relapse occurred. Three of the 7 patients (43%) who experienced a second relapse were still receiving immunosuppressive therapy for their first relapse.

Adverse events. Only moderate and severe treatment-related adverse events (AE) were considered. Twenty-two AE, dominated by infectious complications (73%), were reported in 12 of 33 (36%) patients treated with oral or IV cyclophosphamide. Out of 16 infectious events: 5 pneumonias (31%; one complicated by septicemia), 3 urinary infections (19%; one complicated by septicemia), 2 gastrointestinal infections (12%; one complicated by septicemia), 2 cutaneous infections (12%), and one hemodialysis catheter infection with septicemia. In 3 other cases, septicemia was of unknown origin. The other AE potentially related to treatment included a single episode each of uric ureteral lithiasis, hemorrhagic cystitis, and myelodysplastic syndrome. The myelodysplastic syndrome occurred 16 years after initial diagnosis in a patient with WG who had had 5 relapses. Six patients died (17%), one from infectious complications of the immunosuppressive therapy, the others from unrelated causes.

Acute rise in ANCA titers versus persistently positive ANCA titers. As defined, 28 patients with WG or MPA with a minimum followup of 11 months were included in the analysis. We observed 37 episodes of 1.2-fold acute rises in ANCA titers, 16 of them (in 12 patients) being associated with relapse. For a 1.5-fold increase, 32 episodes occurred in 12 patients, 15 associated with relapse. For a 2-fold increase, 21 episodes occurred in 10 patients, 12 associated with relapse. For a 3-fold increase, 10 episodes occurred in 7 patients, 7 associated with relapse; and for a 4-fold increase, 7 episodes occurred in 6 patients, 6 associated with relapse. All acute rises in ANCA titers occurred within 6 months of relapse or at the time of relapse. At any fold-increase, acute ANCA rises were significantly associated to relapse (Table 3, Fisher's exact test). However, the specificity and positive predictive value of the ANCA rise grew in parallel to the magnitude of the rise. Specificity and positive predictive value culminated at 70% and 95%, respectively, for a 3-fold increase in ANCA titer, and at 86% and 98% for a 4-fold increase. Likelihood was as high as 5.6 and 14.5, respectively. As expected in these conditions, the sensitivity of the test was marginal, 29% and 25%, respectively. At lower fold-increase (1.2- to 2-fold), sensitivity improved, but to the detriment of positive predictive value and likelihood, which were weak (1.8 to 3.2). With regard to persistently positive ANCA titers, relapses occurred in 10 of 24 episodes, allowing calculation of sensitivity and specificity of the test of 42% and 78%, respectively; positive and negative predictive value of 59% and 64%, respectively; and a likelihood and odds ratio of 1.9 and 2.6, respectively. In these conditions, persistently positive ANCA titers were not significantly associated with relapse ($p = 0.14$, Fisher's exact test).

DISCUSSION

The diagnosis and classification of patients with vasculitis is based on clinical and histopathological criteria, and may therefore be susceptible to serious limitations as long as ANCA types are not taken into account. ANCA might certainly add value to diagnosis criteria specificity, and could logically be used in combination with clinical and histopathological findings for establishing a specific diagnosis, as currently considered in the revised Chapel Hill criteria[7]. With regard to the spectrum of clinical expression, prevalence of renal involvement may vary during the course of disease, but in our study globally it did not differ between anti-PR3 and anti-MPO-positive patients with vasculitis, and prevalence reported in the literature is in the range of 75% to 90% of cases[24]. Although they considerably overlap, we observed a profile of clinical characteristics that differentiated the anti-PR3 from the anti-MPO-associated vasculitis clearly (chi-square test, $p = 0.02$). Upper respiratory tract involvement was largely more frequently reported in our anti-PR3 than anti-MPO-positive patients, in agreement with several other reports[25]. This was the case as well for pulmonary and joint involvement in the WG group, although others have reported comparable frequencies[26]. Skin and nervous system involvement was found, in contrast, more frequently in MPA than in WG patients. Concerning demographic characteristics, we found a preponderance of male patients with anti-PR3 (M/F ratio 1.3 to 1.9) and of female patients with anti-MPO-associated vasculitis (M/F ratio 0.3 to 0.8) as described[24,25]. AAV primarily affects older patients, although age range can also include pediatric patients. In our study, we found, like others, that

Table 3. Statistical analysis of ANCA titers, classified as acute rise or persistent elevation.

ANCA Titer	Sensitivity, %	95% CI	Specificity, %	95% CI	PPV, %	95% CI	NPV, %	95% CI	Likelihood	p, Fisher's test
Acute rise, fold-increase										
×1.2	67	45–84	64	50–76	43	27–61	82	68–92	1.84	0.015
×1.5	63	41–82	71	57–82	47	29–65	82	69–91	2.13	0.007
×2.0	50	29–71	84	73–93	57	34–78	80	68–89	3.22	0.002
×3.0	29	13–51	95	86–99	70	35–93	76	65–86	5.64	0.006
×4.0	25	10–47	98	91–99	86	42–100	76	65–85	14.5	0.002
Persistent elevation	42	22–63	78	60–90	59	33–82	64	47–79	1.91	0.14

PPV: positive predictive value; NPV: negative predictive value.

patients with anti-MPO antibodies were older than those with anti-PR3-associated vasculitis[24,25]. However, as a general caveat, we emphasize the retrospective design of our study, the obvious absence of randomization of the study, and potential lack of comparability between the 2 disease categories.

Frequency of relapse largely depends on the definition, treatment regimen, and duration of followup. Relapse usually occurs when immunosuppressive treatment dose is reduced or interrupted, usually within the first 2 years after diagnosis[6]. Most reports of patients with AAV show a roughly 2-fold increase in relapse rate in patients with WG[22,27] as compared to patients with MPA or renal-limited vasculitis[28,29]. In our study, we confirmed this significant trend (relapse rate 74% vs 33%; p = 0.039). Since most patients were not under immunosuppressive maintenance therapy at the time of their first and second relapse, relapse rate was weakly influenced by concomitant therapy. Our data, however, strongly support the administration of 3-week-interval pulsed cyclophosphamide in patients with WG and the current practice recommendation of immunosuppressive consolidation (with azathioprine, for instance) after this induction phase with cyclophosphamide[30].

Our study aimed to examine the prognostic potential of an acute rise in ANCA titer, versus a persistently positive level of ANCA. Interestingly, when acute rise in ANCA titer was stratified in fold-increases to the most recent ANCA titer and related to relapse rate, 3- or 4-fold increases were strongly predictive of a clinical relapse (Table 3). However, sensitivity was low and false-negative rate was particularly high (71% and 75%, respectively). In contrast, the positive predictive value and likelihood of a 1.2- to 2-fold increase in ANCA titer was rather weak. This strongly suggests that a marked acute rise in ANCA titer lends support to prescribing immunosuppressive therapy. However, in view of the high false-negative rate of ANCA, careful monitoring of clinical expression is mandatory prior to making a decision to treat. As reported by others, persistently positive ANCA titers as a predictive factor did not reach a level of significance in our study.

This observation is reminiscent of multiple attempts to correlate various other autoimmune markers with prediction of disease activity or of disease-specific organ failure. This was the case for anti-dsDNA and more recently for antinucleosome antibodies; results have often been controversial and vary widely from one study to another[31-33]. In most cases, a rise in measures such as anti-dsDNA antibodies is observed more often at the time of disease activation rather than in advance of it[32]. This, however, does not exclude a role for autoantibodies as markers of a potential association with disease-specific organ failure or disease activity, such as anti-dsDNA with lupus nephritis or antinucleosome antibodies with SLE activity[34]. With respect to ANCA, the association with clinical relapse as determined by IF or expressed as anti-PR3 or anti-MPO antibodies varies widely, from strong (more than 75%[5,10,11,14,18,21,35,36]), to moderately high, to weaker (25%–75%[12,13,19-21,37]), as recently reviewed[38,39].

The usefulness of ANCA monitoring in WG was first studied by Tervaert, *et al*, who found titers correlated closely with disease activity and predicted all 17 relapses in a prospective study[40]. In a subsequent study in WG patients, the same authors were able to prevent relapses by initiating treatment on the basis of the ANCA rise[14]. Most other successive studies were much less conclusive, not only in predicting relapses in WG but also in MPA, Churg-Strauss syndrome, and renal-limited vasculitis, based on IF or ELISA. The additional measurement of a rise in the IgG3 subclass increased positive predictive value of a rise in ANCA from 71% to 91% but, given its low sensitivity, was of little clinical utility[18]. Despite a significant association between rising ANCA titers and relapse, Hoffmann, *et al* estimated that about one-third of their patients would not relapse during the followup period and would thus have been treated unnecessarily[15]. In these circumstances, aggressive therapy with immunosuppressive agents would of course be unacceptable. However, important discriminative potential was recently demonstrated for anti-PR3, since persistent positivity was associated in these patients with a higher risk of relapse after switching to azathioprine as maintenance therapy[41].

Our study reinforces the weak predictive value of marginal acute increases in ANCA titers, although the predictive value of marked enhancement in titers reached levels of predictive value that might help in therapeutic decision-making. Further, persistently positive ANCA titers did not correlate with relapse.

ANCA determinations should be considered as ancillary measures for patient clinical monitoring, and introduction of immunosuppressive therapy should reasonably be based on clinical and laboratory markers, as well as a patient's previous clinical course.

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